

CLAIMS

1. The use of an organic solvent selected from 2,2,2-trifluoroethanol, ethylene glycol or ethylene glycol dimethyl ether for enhancing formation, potential formation, fluorescence and/or detection of an exciplex.
2. The use as claimed in claim 1 wherein the solvent is 2,2,2-trifluoroethanol.
3. A method of analysis involving detection of an exciplex in a medium containing exciplex forming partners, the method comprising photoirradiating the medium at the appropriate wavelength and detecting for formation of an exciplex characterised in that on photoirradiation the medium contains an organic solvent selected from 2,2,2-trifluoroethanol, ethylene glycol or ethylene glycol dimethyl ether.
4. A method as claimed in claim 3 wherein the medium is a liquid medium and on photoirradiation contains more than 30%, e.g. more than 50%, by volume of said solvent.
5. A method as claimed in claim 4 wherein the liquid medium contains 60% to 99% by volume of the solvent.
6. A method as claimed in any one of claims 3 to 5 wherein the solvent is 2,2,2-trifluoroethanol.
7. A method of analysing a sample to determine the presence or otherwise therein of a target polynucleotide sequence, the method comprising
  - (a) treating the sample under hybridising conditions with

- i) a first polynucleotide probe labelled with a first exciplex partner moiety able on photoirradiation to form an exciplex with a second exciplex partner moiety, and
- ii) a second polynucleotide probe labelled with the second chromophoric moiety, said first and second probes being adapted to bind to mutually exclusive regions of said target sequence such that said moieties are able to form said exciplex which is detectably different from the first and second moieties,

(b) effecting photoirradiation to cause exciplex formation, and

(c) detecting for formation of the exciplex

characterised in that the sample when irradiated contains an organic solvent selected from 2,2,2-trifluoroethanol, ethylene glycol or ethylene glycol dimethyl ether.

8. A method as claimed in 7 wherein the sample on photoirradiation comprises an admixture of water or buffer and the solvent.

9. A method as claimed in claim 7 or 8 wherein the sample on photoirradiation comprises more than 30% by volume of the solvent.

10. A method as claimed in claim 9 wherein the sample of photoirradiation comprises more than 40% by volume of the solvent.

11. A method as claimed in claim 10 wherein the sample on photoirradiation comprises more than 50% by volume of the solvent.

12. A method as claimed in claim 11 wherein on photoirradiation the sample comprises at least 70% by volume of the solvent.

13. A method as claimed in any one of claims 9 to 12 wherein on photoirradiation the sample comprises a maximum of 80% by volume of the solvent.
14. A method as claimed in any one of claims 7 to 13 wherein the solvent is 2,2,2-trifluorethanol.
15. A method as claimed in any one of claims 7 to 14 in which, prior to step (a) the sample is heated to destroy any secondary structure.
16. A method as claimed in any one of claims 7 to 15 wherein after step (a) the sample is heated and then cooled prior to exciplex formation and detection.
17. A method as claimed in claim 16 wherein said heating after step (a) is to a temperature at which the probes, if hybridised to a target polynucleotide sequence, are denatured from the target sequence.
18. A method as claimed in claim 16 or 17 wherein said heating after step (a) is to a temperature not exceeding 90°C, preferably not exceeding 80 °C.
19. A method as claimed in claim 18 wherein said after step (a) is to a temperature not exceeding 70 °C.
20. A method as claimed in claim 19 wherein said after step (a) is to a temperature not exceeding 60 °C.
21. A method as claimed in claim 20 wherein said after step (a) is to a temperature not exceeding 50 °C.
22. A method as claimed in claim 21 wherein said after step (a) is to a temperature not exceeding 40 °C.

23. A method as claimed in any one of claims 7 to 22 wherein the first polynucleotide probe is labelled at its 5' end with the first exciplex partner moiety.
24. A method as claimed in any one of claims 7 to 23 wherein the second polynucleotide probe is labelled at its 3'-end with the second exciplex partner moiety.
25. A method as claimed in any of claims 7 to 24 wherein the first and second exciplex partner moieties are bonded to the first and second polynucleotide probes respectively by linkers.
26. A method as claimed in any one of claims 7 to 25 wherein the exciplex forming partners comprise the pyrenyl group as a first partner and a second partner which comprises at least one aromatic ring.
27. A method as claimed in claim 26 wherein the second partner is a fused ring system.
28. A method as claimed in claim 27 wherein the second partner is provided with at least one electron donating group.
29. A method as claimed in any one of claims 24 to 28 wherein one of the probes has attached thereto the 1-pyrenyl-methylamino group and the other probe has attached thereto either the 2-(N'-methyl-N'-naphthalen-1'-ylamino)ethylamino group or the 2-(N'-naphthalen-1'-ylamino)ethylamino (MMN) group, said groups providing the exciplex partner moieties.
30. A method as claimed in claim 25 wherein the combination of one of the exciplex partner moieties and its associated linker group is the 2-(N'-methyl-N'-naphth-1"-ylamino)ethylamino group and the combination of the other exciplex partner moiety and its associated linker group is the pyren-1-yl-methylamino group.

31. A method as claimed in any one of claims 7 to 29 wherein the oligonucleotide probes are capable of binding to the target nucleic acid such that there is at least one base of that target between the adjacent 3' and 5' ends of the probes as bound to the target.
32. A method as claimed in claim 31 wherein there is one to three bases of the target between the adjacent 3' and 5' ends of the probes as bound to the target.
33. A method as claimed in anyone of claims 7 to 32 wherein at least one of the probes has at least one base mismatch as compared to the polynucleotide sequence.
34. A method as claimed in claim 33 wherein at least one of the probes has one or two base mismatches as compared to the target polynucleotides sequence.
35. A method as claimed in any one of claims 7 to 34 wherein the target polynucleotide sequence comprises DNA
36. A method as claimed in any one of claims 7 to 34 wherein the target polynucleotide sequence comprises a natural nucleic acid and/or an analogue or derivative of such a nucleic acid.
37. A method as claimed in claim 36 wherein the nucleic acid analogue is PNA or LNA.
38. A method as claimed in any one of claims 7 to 37 wherein the target polynucleotide sequence comprises RNA
39. A method as claimed in claim 38 wherein the first and second probes are DNA probes.
40. A method as claimed in any one of claims 7 to 38 wherein the probes are DNA, RNA or analogues or derivatives of a nucleic acid.

41. A method as claimed in claim 40 wherein the probes comprise LNA or PNA.
42. A method as claimed in any one of claims 7 to 38 wherein the probes contain a mixture of at least two of DNA, RNA and analogues or derivatives of nucleic acid in their sequence.
43. A method as claimed in any one of claims 7 to 42 wherein the target and probe strands are free in solution.
44. A method as claimed in any one of claims 7 to 42 wherein at least one (but not all) of the target nucleic acid and/or at least one of the probes is immobilised with there being also at least one of the moieties free in solution.
45. A method as claimed in claim 44 wherein immobilisation is on a solid or liquid substrate.
46. A method as claimed in claim 45 wherein immobilisation is on a 'chip', microarray, a nanoparticle or other surface.